PB-239 840

INDUSTRY SURVEY OF TEST METHODS OF POTENTIAL HEALTH HAZARD

WOODARD RESEARCH CORPORATION

PREPARED FOR
ENVIRONMENTAL PROTECTION AGENCY

NOVEMBER 1974

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. PB 239 840
EPA-560/5-75-002		

4. TITLE AND SUBTITLE	5. REPORT DATE
Industry Survey of Test Methods of Potential Health Hazard	November 1974
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Geoffrey Woodard, Ph. D.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Woodard Research Corporation 12310 Pinecrest Road Herndon, Virginia 22070	2LA328
	11. CONTRACT/GRANT NO.
	68-01-2104
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Toxic Substances U. S. Environmental Protection Agency Washington, D.C. 20460	FINAL
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Nine companies selected as representing a cross section of prominent producers of chemicals identified through four-digit Standard Industrial Classification (SIC) codes were surveyed with respect to methods used to assess toxicological and environmental properties of new and existing chemicals.

Four progressive levels of investigational effort depending upon extent, frequency, and nature of chemical use are identified: single or infrequent exposure; occasional low-level exposure; frequent low-level, occasional high-level exposures; and frequent high-level, general consumer, unavoidable exposures. Factors influencing the decisions regarding the level(s) of investigation needed or the initiation of the next higher level series of studies are explored. No mathematical formula has emerged to specify the level of testing required. A chemical-by-chemical assessment by competent pharmacologists, toxicologists, and chemists is still needed. Lack of a mechanism to trigger expanded levels of testing is a serious problem.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
	test methods chemical assessment industrial testing health hazard levels of testing	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release unlimited	unclassified	96
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	4.75

EPA Form 2220-1 (9-73)

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November 1974

INDUSTRY SURVEY OF TEST METHODS OF POTENTIAL HEALTH HAZARD

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This report has been reviewed by the Office of Toxic Substances, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

The following article, "Toxicology: Cost/Time", by Gehring et al, is reprinted from Fd. Cosmet. Toxicol. 11: 1097-1110 (1973) by permission of Pergamon Press.

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ABSTRACT

Many new and existing industrial chemicals enjoy uses that are either not regulated or are inadequately regulated under State or Federal law in order to prevent possible damage to man and his environment. The Toxic Substances Control Act concerns itself with this area of chemical use. Regulations to implement this act when it becomes law will be promulgated. In order to design appropriate regulations, it is necessary to have a knowledge of the state-of-the-art as practiced by the chemical industry at the present time for evaluation of potential adverse health and environmental effects of new and existing chemicals.

Nine companies selected as representing a cross section of prominent producers of chemicals identified through four-digit Standard Industrial Classification (SIC) codes were surveyed with respect to methods used to assess toxicological and environmental properties of new and existing chemicals.

Four progressive levels of investigational effort depending upon extent, frequency, and nature of chemical use are identified:

- Single or infrequent exposure - Acute and irritation tests
- Occasional low-level exposure - Short-term repeated dose, sensitization, fish and bird studies

- o Frequent low-level, occasional high-level exposures - Teratology, mutagenicity, metabolism, biodegradation, environmental transport studies
- o Frequent high-level, general consumer, unavoidable exposures - Reproduction, lifetime, carcinogenic, environmental fate, food chain studies

Factors influencing the decisions regarding the level(s) of investigation needed or the initiation of the next higher level series of studies are explored. No mathematical formula has emerged to specify the level of testing required. A chemical-by-chemical assessment by competent pharmacologists, toxicologists, and chemists is still needed.

Lack of a mechanism to trigger expanded levels of testing is a serious problem.

This report was submitted in fulfillment of Contract No. 68-01-2104 by Woodard Research Corporation under the sponsorship of the Environmental Protection Agency. Work was completed as of May 15, 1974.

ACKNOWLEDGMENTS

Initial company contacts, selection of SIC codes, and computer sorts were conducted primarily by Applied Management Sciences (AMS), 962 Wayne Avenue, Silver Spring, Maryland 20901, subcontractors to Woodard Research Corporation. Mr. Locke Bogart and/or Mr. Herbert Cantor of AMS also participated in the majority of the site visits.

I. CONCLUSIONS

1. The chemical industry has developed policies and operating procedures that assure no new chemical will reach the marketplace without some degree of testing for potential toxicological and environmental hazard.

2. As the use of a chemical already existing increases, there is essentially no mechanism that triggers expanded toxicological and environmental testing. Such expanded testing when it occurs is nearly always in response to a reaction to some adverse finding outside the company.

3. Four progressive levels of investigations of new chemicals have been identified that depend upon the extent, frequency, and nature of use. Such testing programs have been influenced strongly by requirements and guidelines for testing regulated chemicals.

4. A substantial effort is maintained by industry to control or regulate the purity of raw materials or intermediates used in chemical synthesis. The presence of toxic or potentially toxic impurities is also tightly controlled once these have been identified.

5. The development of a simple, inexpensive, and reliable toxicological screen is needed to bridge the gap between single-dose acute studies and long-term repeated dose studies to serve as an early warning of potential hazard.

6. Not unique to this survey is the observation that reliable and less time consuming systems to detect multigeneration and carcinogenic effects are highly desirable.

7. Tests that might be most predictive in the detection of potential mutagenicity and environmental hazard are still being developed. Such development needs to be encouraged by moving cautiously in establishing rigid protocols or guidelines.

II. RECOMMENDATIONS

In order to provide the maximum protection to man and his environment against the hazardous use of both new and existing chemicals on the one hand, and on the other to minimize the financial burden to the manufacturer and eventually the consumer and taxpayer, it is recommended that:

- Screening methods be developed that are inexpensive and reliable as early warning tests for potential long-range or subtle hazards. These would bridge the gap between single-dose and long-term (approaching lifetime) and multigeneration reproduction studies.

- Development of less expensive and time consuming tests for carcinogens be encouraged.

- Latitude be permitted in test protocols for those areas not yet well standardized, particularly
  - o Mutagen tests
  - o Environmental damage tests

  Establishing rigid protocols for these areas at this time would tend to inhibit additional basic research.

- When a manufacturer is required to file information under a Toxic Substances Control Act, each filing be accompanied by an updated outline of company procedure and policy in assessing toxicological and environmental hazards. There can be no greater safeguard than a thoughtful review by competent personnel abreast of the current state-of-the-art.

- A mechanism be devised to signal the need for expanded toxicological and environmental testing. Such a mechanism should take into consideration

  - o Gross amounts produced
  - o Maximum and mean concentrations resulting from use at both normal and abuse levels
  - o Chemical stability and degradation products
  - o Currently known toxic properties

  Registration at definite intervals could require such an analysis be made for each new filing.

III. INTRODUCTION

Of the new chemical entities that are being developed to the point of commercial products, the majority are regulated by existing Federal legislation. Regulations governing the introduction to the market place of food additives, pesticides, drugs, medical devices, and cosmetics are well known. Legislation regarding the transportation of hazardous substances, the safety of household substances, the safety of surface finishes, and flame-retardant requirements has also resulted in Federal regulations for the evaluation of the toxicology of many chemicals or chemical formulations. Finally, legislation designed to protect the factory worker is increasing the regulation of chemical intermediates. Experience with these regulations will effect profoundly any new toxic substances controls.

In spite of all of the existing regulatory practices with respect to the toxicological and environmental evaluation of chemicals, some chemicals can be identified that are not regulated or are inadequately regulated to protect both man and his environment.

Protection is needed in some cases against product misuse, in some cases against overuse, and in a few cases against any use that would expose man or his environment to appreciable quantities.

Examples of chemicals that fall within the definition of this study are:

- Industrial chemicals ordinarily used in the production of other chemicals, formulations, or polymerics
- Industrial solvents, dry cleaning liquids
- Propellants
- Fuels
- Flame retardants
- Heat transfer materials
- Pigments other than for foods, drugs, and cosmetics
- Fertilizers

The Toxic Substances Control Act when finally enacted is intended to cover these chemical entities whether currently in use or being introduced for the first time. To prepare regulations for the orderly enforcement of this legislation requires a knowledge of the practices being used currently within the industry to assess potential toxicological and environmental hazards of the use of such chemicals.

The purpose of this study then was to establish, as it exists in the industry, the current state-of-the-art for the assessment of potential hazards to man and the environment resulting from the introduction of new or existing chemicals. Of necessity this has involved the comparative evaluation of screening test methods, policies, and procedures used. To accomplish this objective, a sampling of the major chemical industries was undertaken and information based upon site interviews, interviews

with key personnel, public information documents, publications in the scientific literature, and activities of several trade associations was compiled. From this compilation, the ensuing evaluation has been prepared.

IV. METHODS EMPLOYED

SAMPLE SELECTION PROCESS.

Selection of Organizations to be Considered:

As a basis for a good cross section of the chemical industry, the Standard Industrial Classification four-digit code was studied. From this review 40 four-digit classifications were chosen that from their descriptions appeared to include those companies that had the capacity for producing chemicals subject to the Toxic Substances Control Act. These classifications are as follows:

3312	Blast furnaces and steel mills
2911	Petroleum refining
2841	Soap and other detergents
2819	Industrial inorganic chemicals, NEC (Not Elsewhere Classified)
2824	Organic fibers, noncellulosic
3339	Primary non-ferrous metals, NEC
2812	Alkalies and chlorine
3334	Primary aluminum
2823	Cellulosic man-made fibers
3241	Cement, hydraulic
2681	Gum and wood chemicals
2821	Plastics materials and resins
3292	Asbestos products
2851	Paints and allied products
2813	Industrial gases
2871	Fertilizers

2873	Phosphates (transferred from 2874)
2842	Polishes and sanitation goods
2893	Printing ink
2895	Carbon black
2816	Inorganic pigments
2899	Chemical preparations, NEC
3255	Clay refractories
3341	Secondary nonferrous metals
2865	Cyclic intermediates and crudes
2818	Industrial organic chemicals, NEC
2992	Lubricating oils and greases
2843	Surface active agents
2891	Adhesives and gelatin
2951	Paving mixtures and blocks
2822	Synthetic rubber
3251	Brick and structural clay tile
3253	Ceramic wall and floor tile
2892	Explosives
3333	Primary zinc
3332	Primary lead
2952	Asphalt felts and coatings
2999	Petroleum and coal products, NEC
3259	Structural clay products, NEC
3331	Primary copper

A listing of all companies by each SIC code number was obtained from Dun & Bradstreet and was computer sorted to obtain the name of the major one or two companies for each

classification. This listing was cross-checked against rosters of professional societies or attendees at scientific meetings that identified industry supported personnel dealing in the assessment of toxicological and environmental effects of chemicals. An additional 23 companies were so identified. It is believed that the resulting 62 companies so selected perform a significant industry role in the assessment of the toxicological and environmental effects of chemicals both regulated and nonregulated. Both direct and indirect interaction with trade associations such as MCA (Manufacturing Chemists Association), PMA (Pharmaceutical Manufacturers Association), CTFA (Cosmetic Toiletry and Fragrance Association), and standards groups such as ASTM (American Society for Testing and Materials) have provided additional validation of the above selected industrial samples.

Initial Contact Procedure:

The initial contact with each company was made by telephone to an individual that in most cases had professional qualifications as a toxicologist or industrial hygienist. The purpose of the study to be undertaken was explained and a request made for each company so contacted to participate in the study. Nearly every company contacted expressed willingness to cooperate (in some cases subject to review by general counsel or management).

Follow-up Requests:

Exceptions to cooperation was based on the claim that the

company produced no chemicals subject to the pending legislation. One company agreed at first to cooperate but later declined on the basis that their information would be forwarded directly to EPA. Since the personnel involved are personally known to the writer of this report, there is no reason to believe otherwise.

Selection of Final Participants:

Because of federal regulations restricting the number of industrial companies to be studied in depth to nine, a list of 18 companies, a primary and an alternate in each of nine categories, was selected from those companies that had agreed to cooperate. This selection was made during a conference with the Government Project Officer and other EPA personnel. The nine companies whose personnel were interviewed for this report are:

Hercules, Inc.
Exxon Corporation
Proctor & Gamble Company
E. I. du Pont de Nemours & Company, Incorporated
Eastman Kodak Company
Minnesota Mining and Manufacturing
Shell Oil Company
Hoffmann-LaRoche Inc.
Allied Chemical Company

The writer of this report is very appreciative of the time the above companies made available for the study. It

is only regretted that it was not possible to take advantage of all offers of cooperation that were extended. This was not possible because of the OMB restriction previously mentioned. On the other hand, while each company provided procedures unique in detail, the overall policy of each company with respect to toxicological and environmental assessments was aimed at objectives quite uniform within the industry. In the companies studied, these objectives were diligently pursued and matters of product safety had the attention of upper management. Such diligence and attention may not be representative of the entire industry.

VISIT TO SITE AND INTERVIEW.

Preparation of Outline for Interview:

Based upon knowledge obtained over many years' experience in both government and industry in investigations of problems related to the toxicity of chemicals, it was realized that many factors over and above experimental protocols must be considered when evaluating potential environmental and toxicological hazards. In order to obtain information on all important factors, an outline was devised to be used in each interview. After the initial one or two interviews, the outline was followed from memory. Information sought included the following:

- Decision making processes related to toxicological and environmental assessments.

- The commitment made to such assessments in terms of personnel, dollar value or product development.
- Mechanisms for monitoring or controlling marketed products.
- Criteria used to continue or abandon testing.
- Criteria used to limit product distribution or warn users.
- Specific test protocols.
- Criteria used to select appropriate test protocols.
- A listing of warning signs that would indicate more experimental work was required.
- The effect of chemical impurities on safety assessment data.

Conduct of Interview:

Meetings with company personnel and interviews were for the most part informal, although in a couple of sessions a representative from the legal staff of the company was present. Care to avoid judgmental statements was made in order to encourage the free and easy exchange of information. A commitment was made to each company that specific procedures utilized by that company would not be identified with the name of the company in the final report. A few unique test protocols were described.

Collection of Available Documents:

In a number of cases, available published documents were obtained and included items such as annual reports, product information sheets, listing of company products, organization charts, and publications from the scientific literature by company personnel. In a couple of instances, hard copy test protocols were provided. However, in most cases such test protocols were said to be "standard" procedures followed by all investigators.

ORDERING AND SUMMARIZING DATA FOR EACH COMPANY.

Following each site visit, the notes taken by each interviewer (usually two) along with written material, documents, and ancillary information were used to compile an individual "case history" for each company with respect to toxicological and environmental assessment of chemicals. These individual "case histories" form the basis for this report, augmented to some extent by ancillary information as described below.

USE OF ANCILLARY INFORMATION.

A number of other organizations are in various stages of collecting, writing, or standardizing test protocols or guidelines for the toxicological and environmental assessment of chemicals. The ASTM has established Committee E.35 to produce standard protocols for pesticides. PMA in cooperation with FDA is attempting to establish guidelines for drugs. The Society of Toxicology along with the

Association of Official Analytical Chemists (AOAC) has a joint project to establish general guidelines for toxicological testing.

V. EVALUATION OF THE PRESENT INDUSTRY PRACTICES

DECISION MAKING PROCESSES.

Decisions with respect to toxicology and environmental effects of chemicals include resolutions of the following questions:

- When to initiate laboratory testing.
- How extensive should the laboratory testing be.
- What restrictions should be placed on the use of the chemical as a result of the test.
- When should more extensive testing be done.
    - o What criteria can be set up to determine the time for expanded investigations.
    - o What safeguards can be instituted to assure that the need for additional testing will be signaled at an appropriate time.
- What impurities may be permitted in the chemical and at what levels must these be held.
- What new applications will require a new review of the toxicological and environmental properties of the chemical.

In all companies cooperating in this effort, these decisions are made by professional toxicologists who would be qualified on the basis of advanced degrees and from

several to many years of experience in toxicology, biochemistry, or medicine. In decisions involving major expenditures or massive chemical exposures, consultants often are used to provide additional perspective. While such decisions are subject to review and must be defended by the toxicology staff, various strictures prevent overriding or ignoring them by other elements of company management and include:

- Formal corporate policy and procedures with a written sign-off by responsible personnel.
- Sign-off by legal counsel.
- Relationships with liability insurance carriers.
- Making product managers or profit center managers accountable for any product liability.

The practices found in the companies cooperating in this effort are representative of those seen elsewhere in companies both large and small that engage the services of a contract toxicology and analytical laboratory.

Decisions Regarding New Products: Decisions regarding new products are made initially when a chemical becomes available to the Product Manager, usually from Central or Division Research, or in some cases from "outside acquisitions." Typically, several candidate materials will be available and the very first decision will be that of the best choice of the candidates. Depending upon intended

end use of the chemical, toxicological and environmental properties will be a greater or lesser factor in the final choice of the compound to be developed. Ordinarily, these decisions follow joint agreements made by marketing, engineering, production, product application, and safety personnel.

Once the most promising candidate compound is selected, laboratory testing programs are established, the extent of which is determined by:

- Proposed end use - industrial chemical, consumer product, structural components, energy transfer
- Extent of human and environmental exposure - minor, visible, massive
- Duration of exposure - infrequent, occasional, often, daily
- Relationship to chemical structures having known toxicological or environmental profiles
- Chemical properties - stability, metabolic products, degradation products, impurities, binding characteristics
- Physical properties - volatility, radiation adsorption, particle size

These considerations lead to several possible levels of laboratory and field experimentation as will be discussed later.

Decisions Regarding Existing Products: Decisions regarding existing products with respect to assessment of toxicological and environmental properties are the least well defined of all decisions in safety evaluation. Such decisions, when made, are usually reactionary. A company reacts to:

- Consumer complaints.
- Industrial customer complaints.
- Adverse publications in the scientific literature.
- Governmental regulatory action.
- Unions, employees.

Seldom does the industry anticipate these complaints or adverse findings since no machinery has been set up to trigger the need for additional investigations. As a natural progression in a free market place, the extent of use of an available chemical will remain constant, diminish, or increase. If the use remains constant or diminishes, there is no requirement for additional toxicological or environmental evaluation unless the use pattern changes substantially. The increasing use of a chemical is the primary problem producing situation. A current example of such a problem is vinyl chloride. Its production in the United States has increased from 321 million pounds per year in 1952 to an estimated 5.3 billion pounds per year in 1973 (1). At some point in time, this product growth should have triggered substantially increased attention to

its toxicological and environmental properties. This did not occur. It was only after some adverse publications in the scientific literature that the American industry began to react. Even then, the reaction was not aggressively pursued so that at this writing additional adverse findings in Europe have appeared before positive industry action in America has been made public (2).

Compounds that fall within the category of existing products have provoked the greatest present concern to an increasingly consumer oriented public. The polychlorinated biphenyls, fluorocarbons, DDT, vinyl chloride, asbestos, and certain halogenated solvents are such examples. The use in each case began with a few thousand pounds which could be shown to be safe. Current usage has grown in each instance to millions to billions of pounds. This increased usage should have triggered within the industry the development of new toxicological and environmental testing programs. This did not occur. It remained for scientists in the academic community to become concerned and to seek Federal support for programs to assess the possible effects of the massive use of such chemicals. The possibility that there exists a number of other currently marketed chemicals that have been inadequately studied in relationship to their present level of use is almost certain.

Problems leading to the lack of adequate decision making in the area of existing products include:

- Absence of corporate policy requiring reexamination of toxicological and environmental effect profiles at predetermined production or use levels.
- Loss of proprietary position because the patent protection period has expired. Many companies may then share responsibility.
- Absence of industry-wide monitoring programs that would signal the need for more attention to toxicological and environmental impact.
- Failure of primary producers to monitor changes in user applications.
- Failure of regulatory agencies to recognize increased areas and extent of use. Again using the vinyl chloride example, uses were condoned that far exceed industry's own evaluation of the toxic potential (3).

Decisions Regarding Product Changes: Decisions regarding product changes have been carefully considered by all companies cooperating in this project. In fact, a major effort in each company is in existence to evaluate, monitor, and/or approve of any changes in sources of raw materials, specifications for feed stocks, or new methods of synthesis. The need for such attention has been highlighted by the existence of the dioxins in 2,4,5-T(4) and bis-chloromethyl ether contamination of chloromethyl

methyl ether (5). The dioxins are among the most toxic substances known and are teratogenic at very low levels of exposure. The presence of bis-chloromethyl ether may be responsible for cancers reported in workers exposed to chloromethyl methyl ether. Uncertainties in historic supplies and spot shortages of material have made it necessary to consider alternate sources of raw materials and intermediates. These new sources offer supplies that invariably differ from traditional supplies with respect to impurities. These may be various heavy metals and/or organic substances with known toxic properties. It then becomes a task for the industrial toxicologist to determine whether or not the new source is satisfactory in light of previously published information. Obviously, this can become a formidable effort in multiproduct companies. Frequently, it is necessary to consider not only the intrinsic toxicity of the impurities, but also the influence these impurities may have on:

- Catalytic degradation, hydrolysis, or conversion.
- Production of new impurities in synthetic processes.
- Physical properties of the finished product.

These problems are particularly important in the production of pharmaceuticals, polymers, and pesticides.

DEVELOPMENT AND USE OF SPECIFIC EXPERIMENTAL METHODOLOGY.

From interviews with company toxicology personnel and reviews of stated policy, four levels of experimental test-

ing have been identified that correspond to the same number of levels of exposure of either man or his environment. These levels are derived from consideration of length of exposure, extent, avoidable or not, numbers of people at risk, and the portion of the environment exposed. While there may be some overlap, depending upon unique conditions of exposure for a particular chemical, testing procedures may be grouped as follows:

Level I Exposure:

This originally occurs when the chemical is still in laboratory or pilot plant production where a limited number of persons may be exposed. Later, this level also includes the single or infrequent chemical contact through accidental splash, spill, or container rupture. Trivial exposures such as might be experienced by laboratory personnel developing potential end uses are considered here. For this level, the studies usually conducted are:

Single Dose Studies

- o Oral LD_{50}
- o Emetic potential
- o Inhalation LD_{50}
- o Dermal LD_{50}

Irritation Studies

- o Eye, mucous membrane
- o Dermal
- o Corrosiveness

Level II Exposure:

As more persons become involved in the production or industrial use of the chemical where the exposure rates are low

or where high concentrations can be avoided, or where precautionary procedures can be employed, toxicological investigations are expanded to include:

10- to 90-Day Studies By	Sensitization Studies
o Oral	o Dermal - guinea pig
o Inhalation	o Dermal - man
o Dermal routes	o Inhalation - guinea pig

Environmental Behavior

- o 96-hour fish LD_{50}'s
- o 5- to 7-day bird toxicity
- o Half-life determinations in water, soil, etc.
- o Biological or chemical oxygen demand (BOD, COD)

Level III Exposure:

As more and more individuals become exposed through occupational, hobby, repair, or incidental chemical contact, this level has been reached. This includes through the hobbiest, repairman, or craftsman some limited consumer exposure. Frequent low-level or occasional high-level exposures of substantial populations usually are also included within this definition. Studies progressively pursued are:

Teratology
Mutagenicity
Animal or human metabolism

Environmental

- o Biodegradation
- o Metabolic or decomposition products
- o Transport mechanisms

Level IV Exposure:

This level is usually defined as occurring when use has become so widespread that nearly everyone is exposed to the chemical or use is such that exposure to it in small amounts becomes unavoidable. Other types falling within the category are frequent high-level or general consumer exposures. Additional studies conducted for this level are:

Reproduction
Carcinogenic
Prolonged or life-time
Environmental

- o Disposition
  - Sewage treatment
  - Incineration
  - Landfill
- o Food-chain accumulation
- o Long-term aquatic/wildlife

Laboratory and field testing usually proceed stepwise from Level I through Level IV, depending upon the degree of exposure to the chemical expected. At each level of exposure described above, some estimate of accidental or abuse usage must be projected. A degree of accidental

or abuse exposure should be allowed for by employing safety factors applied to data from laboratory experiments.

While the degree of exposure estimated by one toxicologist will vary from that estimated by another, there was quite good agreement among those interviewed for the classifications, as defined above. In the event of doubt about which exposure level best describes the end use of a chemical, the testing procedures to be employed for the next higher category may be utilized.

Because of existing Federal regulations regarding the transportation and labeling of chemicals, nearly every new chemical entity produced in commercial quantities will undergo applicable Level I testing procedures. What frequently happens, however, is that the use of a chemical grows so gradually into a Level II category that no one thinks of conducting Level II studies. This pattern repeats itself through Level IV. It should be noted also that very little or no experimentation is conducted on the environmental effects of a chemical in the Level I category. This lack of environmental studies is in keeping with the limited or restricted use that can occur within the definition of Level I exposure.

Typical protocols are appended. However, as a guide a general description is given here.

Level I:

Single Dose Studies - Single dose studies are conducted in one or more species in decreasing order; rats, mice, rabbits, guinea pigs, dogs, cats, monkeys, domestic (economic) animals. Several dose levels are employed using one to ten animals per sex per dose so that an LD_{50} with 95% confidence limits can be calculated at least for a rodent species. The most applicable route of administration is used, most often oral, dermal, or inhalation. If dogs, cats, or monkeys are used, the emetic potential will be apparent. Animals are observed for seven or more days following exposure and animals that die and representative survivors at the end of the observation period are examined for signs of organ damage at necropsy. Close attention to pharmacologic and toxic signs in such experiments is stressed.

Irritation Studies - Irritation studies are most frequently conducted in the eye and on the skin of the rabbit. For some purposes, the guinea pig may be immersed in the material under test. Also, a specific evaluation of the corrosiveness of the material to the trachea or hamster cheek pouch may be needed where exposure to children becomes a possibility. From three to ten animals (most commonly six) are used for each exposure condition or concentration. Some scheme of describing in a quantitative fashion any reaction seen is ordinarily employed.

Level II:

Repeated Dose Studies - Repeated dose studies of 10 to 90

days in duration are usually conducted in rats for oral and inhalation routes of administration, and in rabbits for the dermal route. Dogs, monkeys, or cats are also employed when a non-rodent test species is desired. At least four groups of animals are used with one group being an untreated control and the remaining groups given dose levels ranging from no (or marginal) effect to a maximum tolerated level showing definite signs of toxicity. Group sizes are most frequently 3 or 4 of each sex for non-rodents, 10 to 15 for rats, and 4 or 5 for rabbits. Studies of 10 to 14 days duration for screening purposes utilize smaller groups. Parameters for study include weight changes, hemograms, clinical chemical tests, changes in behavior, and appearances of the eyes, coat, and mucous membranes. At termination of the studies, histopathological examination of the organs and tissues is made in order to determine most probable target organs.

Sensitization Studies - Sensitization studies are conducted first in guinea pigs and secondly in man to estimate the potential of the chemical to elicit an eczematous sensitization following repeated exposure. Ten to 25 guinea pigs and 50 to 200 people are most often used per compound.

Environmental Impact - Environmental impact studies are first encountered in Level II but probably more often in Level III. These studies are confined to screening studies using Daphnia, fingerlings of available local fish, and

Chlorella. Such screening studies are not used industry-wide and tend to be company specific and remain basically exploratory in nature. Standard protocols that are followed consist of:

- o BOD
- o COD
- o 96-hour LC_{50}'s in one or more species of fish
- o A 5- to 7-day toxicity study in one or two species of birds such as bobwhite quail and mallard ducklings.

These protocols are described in ASTM publications and in EPA guidelines for pesticides.

Chemical studies may also be made that are helpful in predicting environmental impact and these include:

- o Oil/water distribution coefficients or, more often, solubilities in water and a variety of nonaqueous solvents
- o Hydrolysis rates
- o Stability studies using exaggerated conditions of heat, humidity, and/or light under acid, alkaline, and neutral conditions.

Level III:

Level III studies generally include several of the following:

Teratology Studies - These studies are done most often in rats but also in hamsters, mice, and rabbits, and on rare occasions in other species such as dogs, monkeys, or domestic animals. The basic study involves administration of a test compound during the gestation period corresponding to the days of organogenesis. Fetuses are removed usually one or two days prior to normal birth. Some of these are cleared and stained in order to permit examination of skeletal development. The remainder are preserved to allow subsequent examination of soft tissues for any abnormal development. Groups of 20 or so pregnant females for rodents and 5 to 10 for non-rodents are employed using two or more dose levels, a control level and sometimes one level of a reference compound.

Mutagenicity Studies - Mutagenicity studies are beginning to be employed with greater frequency in the investigation of new chemicals. A lack of general consensus as to the proper methods for such assessment has hampered the development of effort in this area. Methods most frequently employed include:

- Some version of the Bateman Dominant Lethal test, using either mice or rats.
- A version of the host-mediated assay.
- A system using bacteria, an *in vitro* cell system, or some small organism that multiplies rapidly.

Versions of such test protocols are described by Hollaender (6).

Animal (Human) Metabolism - Animal (human) metabolism studies are being employed frequently at this level particularly since at this point in the exploitation of a chemical it becomes important to compare the metabolic disposition in laboratory animals, in the environment and in man. Such studies provide the basis for refining estimates of the levels of exposure that may be permissable. These metabolism studies generally follow a standard pattern progressing as follows:

- o Absorption, and excretion in animals (possibly including man)
- o Metabolic fate in the animal
- o Metabolic fate in plants or other biotic compartments of the environment.
- o Identification of metabolites, breakdown products, and sites of accumulation in both animals and in the environment.

Environmental Studies - Environmental studies for Level III include those described for Level II if they have not yet been conducted and in addition:

- o Biodegradation in receiving waters, soils
- o Soil and water movement of transport

- Identification of metabolites or decomposition products if not already achieved as noted above.

For most of these studies, standardized protocols are still under development. However, a few selected protocols have been described by ASTM (7) such as for the biodegradation of detergents. Sound analytical chemical procedures otherwise are followed in attempting to answer the questions regarding fate in the environment and the kinetics involved.

Level IV:

Level IV studies generally are employed only as a reaction by the industry to some adverse finding or publicity. A number of monomers in addition to vinyl chloride are under study or a new review. A number of industrial solvents that have been in use for years are receiving renewed attention. One exception to this generalization was the massive amount of work conducted on NTA (sodium nitrilotriacetic acid) prior to any massive human and environmental exposure (8,9). This level of testing for NTA indicates a recognition of the potential adverse effects of the introduction into the environment of massive quantities of a new chemical. The lesson of the phosphate detergent builders was not ignored. The additional studies usually considered in Level IV investigations include all of the following where applicable:

Reproduction Studies - Reproduction studies usually are conducted in rats for three generations. Groups of 20 animals of each sex will be exposed by the appropriate route to various levels of the compound beginning 60 days prior to mating and through gestation and lactation of second litters. Selected animals from second litters will then be used as parents of the second generation. This will be repeated through the second litters of a third generation.

Carcinogenic Studies - Carcinogenic studies are normally conducted in both rats and mice by the appropriate route for the approximate life span of the strains used (usually 18 to 24 months). Two or three dose levels are employed with 35 to 50 animals of each sex per group. Historic data along with an equivalent or even larger group of control animals are required in order to lend validity to the results obtained. Gross and histological examination of the tissues of all animals, both surviving and non-surviving, must be made to avoid possible experimental artifact.

Prolonged or Lifetime Studies - Prolonged or lifetime studies may be employed using non-rodent species to detect chronic organ damage other than cancer. These studies generally extend over 6 to 24 months. In rare instances studies approaching lifetime in dogs and monkeys are, or have been, undertaken. The numbers of animals per group

are usually three or four of each sex. Study parameters include growth, biochemical, hematological, neurological, behavioral, histopathologic, and eye changes. Details of these study parameters are listed in typical protocols appended. Some exceptional studies have used larger numbers of animals. Birth control drugs are studied for seven years in dogs, 10 years in monkeys, with group sizes of 16 of one sex.

Environmental Disposition Studies - Environmental disposition studies include an evaluation of the consequences of the disposal of the chemical, or materials containing the chemical, by one of the avilable methods:

- Waste water treatment systems
  - activated sludge
  - septic
  - oxidation ponds, trenches, filters
- Incineration
- Landfill
- Composting

Commonly employed protocols are not available so that such studies fall more nearly within the category of basic research. Sound analytical chemistry and thoughtful experimental plans are obviously of paramount importance.

Movement Within the Environment - Movement within the environment or mobilization of previously bound or fixed

materials as a consequence of the chemical is also studied at this level. Evidence on the following points is sought:

- Possible buildup through food chain magnification.
- Bioaccumulation by specific plants and aquatic organisms.
- Mobilization of existing toxic substances that have been bound or fixed in bottom muds or in poorly drained soils. These are most often inorganic substances.

MONITORING SYSTEMS:

One critical area that uniformly receives attention by the companies cooperating in this study is that of monitoring their marketed products from the standpoint of safety. This takes the form of:

Consumer Complaints Handling - These arise in several ways and all are handled by the Toxicology Staff:

- Telephone "Hot Line," a nationwide 800 area code number which is attended 24 hours a day, seven days per week, by someone sufficiently trained to give advice or who knows where to obtain it for any emergency related to a chemical exposure.

- Complaint letters containing any remarks regarding ill effects to plants, animals, or man.
- Complaints from marketing or sales personnel--usually from industrial customers, rather than individual consumers.

Marketing Liaison on New Applications - Marketing liaison on new applications is generally managed in either a formal way in a few companies or informally in the remaining companies, by designating a "Toxicology Liaison" individual in each operating division. This person is charged with the responsibility of clearing any question regarding toxicity or adverse environmental effect arising within his division:

- First with the company toxicology and/or environmental staff.
- Secondly with outside consultants or advisors.
- Sometimes through inquiry with a trade association.
- Often through inquiry with his professional counterpart in another company.

Various built-in safeguards are established to assure that such precautions are taken. Such safeguards include:

- Insurance carrier review. Some product liability insurance companies base rates in part on toxicological information available.
- Charges to profit centers for product liability when safety procedures have been ignored or compromised. The profit center may be a company division or a product line production and sales group which has been identified and held responsible for returning a profit to the company.
- Review by legal counsel.

When to Expand Toxicological and Environmental Testing - No company within those cooperating in this study has a system that would automatically trigger an expanded testing program. Some mechanism to bring about this kind of attention to toxicological and environmental problems is yet to be devised, based upon information gleaned in this survey. A variety of factors have contributed to the lack of development of such a system. These include:

- Loss of proprietary position and resulting fragmentation of responsibility for the product produced when patents expire.
- Growth of product use has failed to alert responsible personnel in the absence of any

fixed company guidelines. To establish fixed guidelines would involve some measure of acute, chronic, and incidental or minor exposures, along with an evaluation of the degree and nature of toxic or environmental sequelae. At this point in time no such formula has been advanced, much less tried empirically.

- Because of the foregoing problems, the total extent of use of a chemical ceases to be under review of a single individual. Consequently, the fragmented use of such a material, while being observed in individual situations by competent personnel, lacks the impact of a knowledge of the total exposure. The United States Tariff Commission compiles production data on chemicals that could be used as part of an alerting mechanism.
- Also because of the foregoing situations, the availability of new methods for the assessment of toxicological and environmental effects of chemicals are not followed up by individual company action.

CHEMICAL PURITY:

While specifications for chemical purity have not been discussed in connection with company policy or testing

programs, it is recognized throughout the industry that the chemical identity and purity of any chemical used in toxicological and environmental studies must be specified carefully. Consequently, any study being undertaken currently will be reported in terms of a particular batch, or mix of batches, for which there are available chemical analyses showing:

- o Content of active ingredient
- o Moisture
- o Organic solvent residual
- o Amount and nature of impurities
- o Heavy metal content.

Batches of chemicals exceeding these specifications are not covered by the toxicological and environmental experimental data obtained.

Industry generally has become very sensitive to the problem of impurities, especially since the 2,4,5-T episode and a few less publicized examples, such as the content of beta-naphthylamine in alpha-naphthylamine.

COSTS FOR CONDUCTING TOXICOLOGICAL AND ENVIRONMENTAL HAZARDS ASSESSMENT:

Appended to this report is a copy of a paper (10), which reports a survey of costs for nearly all of the test

methods described for toxicology. Several of the laboratories that participated in the survey have also participated in the survey reported here. In addition, a number of "independent" laboratories participated in the survey so that reported costs are quite well documented. The cost survey was completed in 1972. Consequently, a factor at least equal to the intervening increase in the cost of living index must be applied to these figures to make them applicable today.

Excepting costs of tests on birds and aquatic life contained in the above cited survey, no good estimate of costs were possible for the various environmental test procedures.

VI. IDENTIFICATION OF PROBLEM AREAS IN TOXIC SUBSTANCES EVALUATION

NEW PRODUCTS:

The majority of new chemical entities that become commercially available are not examined beyond Level I tests. The degree or extent to which man or his environment is exposed to such chemicals does not warrant more extensive testing and by the same token the sales volume would not support the costs of more extensive studies. Unfortunately, Level I tests are based upon single or acute exposures and give no clue or warning of the more subtle effects that can follow repeated low-level use. There is a need for a biological screening system that would be predictive or would serve as a warning of possible long-term effects. Such a system need not evaluate long-term effects, but should be capable of signaling possible adverse effects should the chemical enjoy increasing use. The cost of such a test system should compare favorably with other Level I tests.

A 5- to 14-day repeated dose LD_{50} screen is used by two companies in this survey, and by two other companies outside the survey, in an attempt to guard against repeated dose "toxicological surprises." Regulatory requirements for single dose LD_{50}'s and irritation studies

may have inhibited the development of more rational short-term repeated dose studies.

When the projected level of use of a new chemical places it into categories requiring more extensive testing, there is currently available satisfactory methodology to answer known concerns about possible toxicity and environmental effects. These higher level tests, however, are relatively expensive so that they will not be employed until a satisfactory product profitability can be projected. Obviously, if inexpensive but reliable screening tests could be developed, potential toxicological and environmental hazards would be known at a much earlier time.

EXISTING PRODUCTS:

By far the most serious deficiency in present programs for the assessment of toxicological and environmental hazards of a chemical is the lack of any mechanism to trigger expanded levels of testing. The typical case history of a chemical involves the production of relatively small amounts of the chemical, usually less than a tank car full. At this time, Level I toxicity tests are the maximum justified. Availability of the chemical is made known, along with its physical and chemical properties. If use of the chemical catches on within the next several years, and if the manufacturer can maintain some proprietary position, it is likely that Level II

tests will be conducted, but there is no assurance of this. With but a few exceptions, any testing beyond Level II will be done as a reaction to some problem and not as a planned affirmative action. The exceptions have been investigations on chemicals to replace those now known to be troublesome either because of newly discovered toxic properties or potential damage to the environment.

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VIII. APPENDICES

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INDUSTRIAL PROFILES

OF COMPANIES INCLUDED IN SURVEY

	Annual(7) Sales Billions	No. Employees	Toxicology Effort** Inside	Outside
Exxon	25.724	137,000	-	++
E.I. du Pont	5.275	118,400	++	±
Shell Oil	4.883	32,000		
Eastman Kodak	4.035	120,700	++	±
Proctor & Gamble	3.906	47,000	++	±
MMM	2.545	78,900	-	++
Allied Chemical	1.664	33,600	-	++
Hercules	1.154	24,100	-	++
Hoffmann-La Roche	1.827*	39,000*	++	±

* Projected estimates from Fortune Magazine, August 1971 (8)

** Toxicological effort related to experimental laboratory work

Fd Cosmet. Toxicol. Vol. 11, pp. 1097–1110. Pergamon Press 1973. Printed in Great Britain

Toxicology: Cost/Time*

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(*Received 27 June 1973*)

Some type of evaluation of the toxicity of pesticides and the hazard of exposures therefrom has been carried out since the advent of pesticide use. Originally, pesticide manufacturers assumed it their moral responsibility to evaluate hazards and provide directions for safe handling and use. This developed into a legal responsibility as well. Several legislative acts have provided for pesticide regulation and all its ramifications including toxicity and safety evaluation (McCollister, 1965; US Department of Agriculture, 1968).

The Federal Insecticide, Fungicide and Rodenticide Act passed in 1947 and amended in 1959 and 1964 requires registration of all economic poisons prior to distribution in interstate commerce. Economic poisons include a wide variety of agents, pesticides among them. For registration to be obtained, data must be presented which demonstrate that the material is safe when used as directed and effective for the purpose claimed on the label. The Act also states that any residues remaining on food or feed must not exceed the established tolerance level.

The provision for tolerances is the result of the Miller Amendment in 1954 to the Food, Drug and Cosmetic Act. The original Food, Drug and Cosmetic Act, passed in 1906, provided that a food should be deemed adulterated if it contained any added poisonous or deleterious substances. The only exception was when such substances were required in production or could not be avoided in good manufacturing practice. With passage of the Miller Amendment, pesticide chemicals were officially recognized as essential to the production of food, thus providing for the establishment of tolerances in raw agricultural commodities. Tolerances are obtained by submitting to a governmental agency (formerly the FDA, currently the EPA) a petition containing extensive data covering toxicity, metabolism, residue levels, including an analytical method for determining residues on foods, and finally a reasonable basis for supporting the safety of the proposed tolerance.

Another law that has greatly influenced the establishment of tolerances for pesticides is the 1958 Food Additive Amendment to the Food, Drug and Cosmetic Act. This amendment contained the Delaney Clause, which states that no food additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animals, or if it is found, after tests which are appropriate for the evaluation of the safety of the food additives, to induce cancer in man or animals. Many have interpreted this to mean that a zero tolerance must be applied to a pesticide found to produce neoplasms in animals regardless of dose, route of administration or species.

*Presented at the 163rd ACS National Meeting, Boston, Mass., 9–14 April 1972; Symposium—Economics of Pesticides: Current and Future.

The purpose of this presentation is to elaborate on the toxicological studies that are either required or recommended for registration of pesticides, including the establishment of a residue tolerance on food crops. In particular, the costs and time required for completion of the studies will be discussed. The values for cost and time were derived from information supplied by the following toxicology laboratories: Food and Drug Research Laboratories Inc., Dow Pharmaceuticals, Woodard Research Corporation, Mellon Institute, Hazelton Laboratories, Gulf South Research Institute, Bionetics, Diamond Shamrock Chemical Company, American Cyanamid, Eastman Kodak Company, Eli Lilly and Company, The Haskell Laboratory of E. I. DuPont de Nemours and Company, Vick Chemical Division of Richardson-Merrell, The Dow Corning Corporation, the Chemical Biology Research Laboratory of the Dow Chemical Company and Industrial Bio-Test Laboratories. Each participant provided estimates, usually as a range, for conducting the various studies described in a general protocol which was supplied. The time estimates included the time needed to conduct the experiments and prepare the reports.

In the early 1950s, toxicology requirements for registration of a pesticide were generally limited to data from single-dose oral administration and from a subacute study in which the material was fed to rats for a period of 30–90 days, with measurement of a few indicators of toxicity. Even in terms of today's inflated dollars, the cost of these studies would amount to less than $10,000.

The picture is very different today. Three factors have contributed to more rigorous and extensive toxicity testing. First the increased sensitivity of analytical methods for detecting pesticide residues has increased the emphasis and concern over the possible effects of low levels of pesticide residues heretofore undetected. Secondly, there has been an increase in the sensitivity and number of techniques for monitoring signs of toxicity, such as electron microscopy, biochemical methodology and radioisotopes and new test systems such as teratology and mutagenicity testing. Some of these developments allow measurement of different parameters of toxicity or of smaller deviations from normal in test animals, while others merely add bulk. Thirdly, an increased awareness of, and concern for, the impact of pesticides on the environment has led to more extensive studies on non-target species in the environment, whereas previously, toxicology was primarily concerned with the evaluation of hazard to man and to a species upon which the product was to be used.

The cost and time needed for completion of the acute toxicity studies required or recommended for pesticide registration are presented in Table 1. These provide data for preliminary evaluation of handling hazards as well as for characterization of the effects of acute accidental or purposeful exposures to man and to parts of the environment.

The oral LD_{50} is the most common expression of toxicity obtained. This measures the most drastic manifestation of toxicity—that of death. It is a statistically derived expression of the single dose of a material that can be expected to kill 50% of the animals treated. Various species may be used, such as rats, mice, guinea-pigs and rabbits. Along with providing numerical data, this test allows an opportunity for the observation of signs of toxicity in the surviving animals. In some cases, gross pathological and histopathological examinations are performed to reveal the target organs. Acute oral toxicity data, including the LD_{50} and description of observed effects, are recommended on at least two species of laboratory animal. The average low and high estimates of costs quoted by 14 laboratories for these studies were $796 and $1230, with a mean of $1013. A period of 4–5 weeks is required to complete the studies.

The dermal LD_{50} is also required. This is an estimate of the absorption of a material

Table 1. *Cost/time estimates for acute studies*

Test	No. of quotes	Cost		Time required for completion‡ (wk)
		Mean (M$)*	Average range† (M$)*	
Individual tests				
Oral LD_{50}	14	1·01		
Dermal LD_{50}	14	0·65		
Inhalation LC_{50}	12	0·89		
Eye irritation	14	0·26		
Skin irritation	14	0·26		
Avian oral LD_{50}	8	1·88		
Fish LC_{50}	9	0·58		
Crustacean LC_{50}	3	0·62		
Subtotal		6·15	5·16–7·11	6
Plus formulation		12·30	10·32–14·22	12
Plus 1 metabolite		18·45	15·49–21·33	18

*Thousands of dollars.
†Average of low estimates–average of high estimates.
‡If tests are run concurrently.

through the skin in acutely toxic amounts. Rabbits are the usual test animal. Other evidence of toxicity besides death is also noted. The average range quoted for such a study was $536–$758, with a mean of $647. This test requires 4–5 weeks for completion.

The inhalation LC_{50}, also required unless it is not reasonable to expect such exposures to be encountered, is the concentration expected to kill 50% of the test animals when the material is inhaled for 1 hour. This study is conducted in the rat and costs ranged from an average of $750 to $1020, mean $885. The study takes 4 weeks to complete.

In the required eye-irritation studies, the material is applied to the eyes of a rabbit and observations as to the type and severity of damage are made at various times after exposure. The effectiveness of washing the eye following application is also investigated. About 3 weeks are required to complete these studies, which range in average cost between $210 and $315, mean $262.

Skin irritation studies are also conducted on rabbits. The skin is clipped free of hair and either abraded or left intact. Evidence of local irritation, which may vary from mild redness to severe burn, is noted. The average range quoted for these studies was $217–$294, with a mean of $255. Three weeks are required for completion.

Oral LD_{50} data are required in birds as well as laboratory animals. Bobwhite quail and mallard ducks are recommended. These studies take 5–6 weeks to complete and range in average cost from $1528 to $2225, mean $1876.

Acute toxicity studies in fish are also required. The estimates had an average range of $525–$636, mean $580. Completion requires about 3 weeks.

Data on acute toxicity to other forms of aquatic organisms, such as shrimp and other crustaceans, may also be required, costing on average between $500 and $633, mean $617. Only three estimates were obtained. The studies take approximately 2 weeks.

The total cost, then, of these acute studies on a pesticide chemical would range on average from $5162 to $7111, with a mean of $6135. At least 6 weeks would be required for completion, providing the tests were run concurrently. These acute studies are also conducted

on the formulated product, and on major metabolite(s). Thus the total cost for acute toxicity studies would average $18,405, and would require 18 weeks.

The subacute toxicity studies are intended to evaluate the effects of relatively short-term repeated exposures to pesticides (Table 2). Ninety-day feeding studies are required on the parent chemical and on major metabolite(s) in at least two species. These involve the continuous administration of the test material to the animals, usually in their diets, at several dose levels. One of the species has to be a non-rodent, and dogs are most often used. The rodent species used is generally the rat. From 80 to 120 rats and from 16 to 24 dogs are used in these tests and at least three dose levels are fed. There is also an untreated control group. Observations are made regarding growth, food intake, appearance and demeanour, and mortality. Laboratory tests are conducted on urine for evidence of kidney damage and on blood for untoward effects, such as anaemia. Various organ-function tests are conducted

Table 2. *Cost/time estimates for subacute studies*

Test	No. of quotes	Cost		Time required for completion‡ (months)
		Mean (M$)*	Average range† (M$)*	
90-Day/dogs: Parent chemical	14	17·4		
Metabolite	14	17·4		
90-Day/rats: Parent chemical	14	12·8		
Metabolite	14	12·8		
21-Day dermal	11	7·0		
14-Day inhalation	10	6·1		
Reproduction: Quail	4	12·4		
Duck	4	14·8		
Total		100·7	88·7–112·3	13
Additional§		13·2		

*Thousands of dollars.
†Average of low estimates-average of high estimates.
‡Assuming studies on the parent chemical and metabolite are staggered.
§Studies on wildlife under field conditions, neurotoxicity and cataractogenic potential.

by measuring blood levels of certain substances, such as urea and protein and the activity of various enzymes. At termination of the study, selected organ weights are recorded. These usually include the heart, liver, kidney, spleen, testes and brain. Gross and microscopic examinations are conducted on approximately 30 tissues. Additional clinical studies may be conducted, such as blood and brain cholinesterase determinations in the case of organophosphate and carbamate insecticides. The objectives of these 90-day studies are to determine the nature of the effects related to ingestion of the compound and to define the minimum dosage level at which the most sensitive criterion revealed an effect and the maximum dosage at which no untoward changes were detected. The estimated cost of the dog studies ranged on average from $15,795 to $18,967, mean $17,381. The rat studies cost slightly less, averaging between $11,310 and $14,200, mean $12,765. Each study takes about 6 months for completion.

The 21-day dermal study consists of daily applications of the chemical to the skin of rabbits, with observations made for evidence of absorption through the skin in toxic

amounts. Body-weight gain and clinical and pathological parameters are evaluated. This study was estimated to cost on average between $6380 and $7520, with a mean of $6940. Completion requires 3–4 months.

A 14-day inhalation study in rats has been mentioned as sometimes desirable. It evaluates the effect of repeated exposure to pesticides via inhalation, using parameters similar to those discussed for other subacute studies. The average range of costs estimated by ten laboratories was $5590–$6640, mean $6120. The time required for completion is 3 months.

Preliminary evaluation of the effects of pesticides on reproduction in two species of birds is required. Bobwhite quail or pheasant and mallard duck are recommended. Egg production, fertility, hatchability and survival are determined. These studies take from 6 to 7 months or longer to complete. Costs average between $10,000 and $14,750, mean $12,375, for quail and between $12,500 and $17,000, mean $14,750, for mallard duck.

These subacute studies just described, including 90-day studies in two species on one metabolite, range in average costs from $88,690 to $112,290, with a mean of $100,730. At least 7 months would be required for the studies on the parent compound, assuming they were run concurrently. The feeding studies on the metabolite(s) would be conducted later and would take another 6 months. Other studies could be required, depending upon the nature and use of the compound and the results of the studies just described. For example, subacute studies on wildlife under field conditions would be necessary if indicated by LD_{50} data. Data from four sources give an average range of $2850–$3600, mean $3225. About 5 weeks are required for completion. A neurotoxicity study in chickens is required for organophosphates, costing about $1000 (one estimate). Studies for evaluation of cataractogenic potential may be indicated and could cost $9000 (one estimate). The conclusion of these additional tests would bring the average cost of subacute toxicity studies to $113,760.

Metabolism studies (Table 3) are conducted in an attempt to uncover the means by which the animal deals with the chemical. These studies include investigation of which organ(s) metabolize the material and the identification of major metabolites, absorption from the intestine, storage in various tissues, and excretion in urine and faeces. Such studies use some of the more recently developed analytical tools, notably radioisotopes and various types of chromatography and spectrometry. Metabolism studies in rats and dogs are required for pesticides. These investigations can vary considerably in extent, developing into highly complex, expensive and time-consuming efforts. The scope of the studies usually

Table 3. *Cost/time estimates for metabolism studies*

Species	No. of quotes	Cost		Time required for completion (months)
		Average range* (M$)†	Mean (M$)†	
Rat	7	9·6–20·4	15·0	3–12
Dog	7	9·2–20·3	14·7	3–12
Total		18·8–40·7	29·7	3–12‡
Man	5	17·5–24·7	21·1	1–3

*Average of low estimates–average of high estimates.
†Thousands of dollars.
‡If run concurrently.

conducted on pesticides involves a period of 3–12 months and ranges in cost on an average between $9600 and $20,433 for rats, mean $15,015, and from $9200 to $20,266 in dogs, mean $14,733.

Carefully controlled studies may also be carried out to investigate the metabolism of the pesticide directly in man and so allow for more meaningful comparisons with animal models. Subacute toxicity studies must be completed first in order to evaluate the risk involved. The experiments in man usually consist of administration of a single oral dose, followed by identification and quantitation of the parent compound and metabolites in blood, urine and faeces. The average of the range of estimates received from five laboratories was $17,500–$24,666, mean $21,080. Any time from 1 to 3 months or more may be required for completion of investigations in man.

For some pesticides, studies in man may be conducted to determine a threshold level which, if exceeded, will alter a sensitive biochemical activity. This is particularly appropriate for cholinesterase inhibitors. The chemical can be administered to human subjects at low dosages, and the blood cholinesterase activity can be measured as an indicator of physiological response, with minimum risk to the test subjects. Time and money requirements would vary considerably according to the particular design, but these studies may cost $50,000 and could take a year for completion.

Studies revealing possible effects on reproduction or foetal development are required for pesticide registration. Multigeneration reproduction studies (Table 4) are usually conducted in rats, although mice, rabbits or guinea-pigs may also be used. The animals are maintained on various doses of the test chemical throughout the study. The original parent group is allowed to produce two litters and offspring from the second litter are in turn raised as parents for the second generation, from which two litters are produced. A third generation is usually derived in the same manner. Observations are made on each breeding trial regarding fertility, survival of offspring, lactation and any evidence of abnormalities in the foetuses. These studies in rats were estimated to average in cost from $33,360 to $37,460, mean $35,410. Two years or more are required for completion. Studies in the other species mentioned cost about the same.

Table 4. *Cost/time estimates for multigeneration reproduction studies*

Species*	No. of quotes	Cost		Time required for completion (months)
		Average range† (M$)‡	Mean (M$)‡	
Rat	12	33·4–37·5	35·4	24–25
Mouse	8	22·4–27·4	24·9	24
Rabbit	4	26·5–38·8	32·6§	32–35
Guinea-pig	3	27·3–36·6	31·8	30–35

*At least one required.
†Average of low estimates–average of high estimates.
‡Thousands of dollars
§It is anticipated that studies using rabbits would cost 15–20% more than those using rats. The apparent discrepancy is attributed to the fact that laboratories submitting the highest quotes for rats did not submit estimates for rabbits.

Although the multigeneration reproduction test provides for some evaluation of teratogenic potential, separate, more specific, studies are recommended and are usually conducted (Table 5). These studies involve administration of the test material in several doses

Table 5. *Cost/time estimates for teratogenicity studies*

Species*	No. of quotes	Cost: Average range† (M$)‡	Cost: Mean (M$)‡	Time required for completion (months)
Rat	12	7·4–13·5	10·4	4
Mouse	9	5·6–7·5	6·5	4
Hamster	5	7·9–9·4	8·6	4
Rabbit	10	9·2–10·8	10·0§	4
Monkey	4	48·8–63·0	55·9	14–16

*One or two required.
†Average of low estimates–average of high estimates.
‡Thousands of dollars.
§It is anticipated that studies using rabbits would cost 15-20% more than those using rats. The apparent discrepancy is attributed to the fact that laboratories submitting the highest quotes for rats did not submit estimates for rabbits.

to pregnant females during the sensitive period of organogenesis. The foetuses are obtained by Caesarean section prior to the expected parturition date and are observed for evidence of abnormalities. Several species may be used, including the rat, mouse, hamster, rabbit or monkey. The cost data obtained for these studies in rats were $7373–$13,467 for the average range, with a mean of $10,420. Studies in mice and hamsters are in the same price range. Generally, rabbits are a little more expensive, and studies in monkeys are 5–10 times more costly. Tests in all species except monkeys require about 4 months to complete, but at least 1 year is necessary in the case of monkeys.

The long-term toxicity studies are conducted primarily for evaluation of carcinogenic potential (Table 6). Test animals may be the rat, hamster or mouse, although the dog has

Table 6. *Cost/time estimates for chronic feeding studies*

Species*	No. of quotes	Cost: Average range† (M$)‡	Cost: Mean (M$)‡	Time required for completion (months)
Dog (2 yr)	12	75·9–89·2	82·6	29–30
Rat (2 yr)	13	68·5–77·1	72·8	28–29
Hamster (2 yr)	7	45·8–52·3	49·0	28–29
Mouse (18 months)	8	45·2–49·3	47·2	27–28
Total for two species (rat and dog)	...	144·4–166·3	155·3	28–30§

*Two required.
†Average of low estimates–average of high estimates.
‡Thousands of dollars.
§If run concurrently.

been used extensively in the past. Tests in two rodent species are now being suggested, lasting 2 years except for those in mice, which run for 18 months.

Residue data and subacute studies may indicate the necessity for chronic studies also on a metabolite which would double the cost. As currently conducted, these studies follow the same general design as the 90-day studies. More animals are started on the test, at least 25/sex/dose and frequently 40–100/sex/dose, to assure sufficient numbers at termination. Special attention is given to tumour formation, but otherwise clinical and pathological evaluations are similar to those in the subacute studies.

At present, there is not a firm requirement for mutagenicity studies, although they are proposed and are likely to become part of the testing programme (Table 7). The relevance

Table 7. *Cost/time estimates for mutagenicity studies*

Test	No. of quotes	Mean cost (M$)*	Time required for completion (months)
Host-mediated assay	7	1·7	2–3
Dominant lethal	9	8·1	4
Cytogenetic studies	7	7·3	4
	Total . . .	17·1†	4‡

*Thousands of dollars.
†Average range (average of low estimates–average of high estimates), M$ 15·0–19·4.
‡If run concurrently.

of the results for assessing human hazard, however, is still open to question. Three studies are recommended:

Host-mediated assay. This system involves injection of bacterial organisms into the peritoneal cavity of a mammalian host, followed by administration of the test material to the host by a different route. The bacteria are then recovered and examined for the induction of mutants. This study ranges in average cost between $1468 and $1968, mean $1718. Approximately 2–3 months are required.

Dominant lethal test. This involves mating treated male rats, usually each week for 7 weeks after treatment, with untreated females and observing mortality *in utero*. The average range of costs from nine laboratories was $7330–$9000 with a mean of $8170. The time required is approximately 4 months.

Cytogenetic studies. *In vivo* cytogenetic studies consist of examination of the chromosomes of treated animals and sometimes of exposed human beings for aberrations. These studies take about 4 months and cost on average from $6236 to $8380, mean $7310.

The total cost of the toxicological evaluation of a pesticide, then, would range from $306,800 to $375,480 at a minimum, with a mean of $349,800 (Table 8). The studies would take a minimum of 4 years, and more probably 6 years, to complete, depending upon how the programme was scheduled. The addition of various other studies, such as extra subacute investigations, human studies, mutagenicity studies, teratology on another species and chronic studies on a metabolite would bring the total to about $625,600. These are very likely additions and are the rule rather than the special-case exception. This is by no means

Table 8. *Summary of cost/time estimates for toxicity testing*

Test	Mean cost (M$)*
Minimum requirements	
Acute	18·4
Subacute	100·5
Metabolism, rat and dog	29·8
Reproduction, rat	35·4
Teratology, rat	10·4
Chronic feeding, rat and dog	155·3
Total . . .	349·8†
Additional tests	
Subacute	13·2
Teratology, second species	10·0
Human metabolism	21·1
Mutagenesis	17·2
Human toxicology	50·0
Chronic feeding, metabolite	155·3
Total . . .	275·8

*Thousands of dollars.
†Minimum time required for completion, 48 months.

the upper limit, either. In some instances chronic inhalation studies may be required for evaluation of carcinogenicity and these would cost in the range of $80,000–$125,000, bringing the total to almost $750,000.

The interest which the money expended for these studies would draw constitutes an additional substantial cost. Also not considered are the additional costs of administration, consultation and liaison, which may add 20% or more to the cost of a study.

This programme for obtaining acceptable toxicity data on pesticides involves large amounts of time and money. Moreover, the impact of the results is considerable, in that safety factors and tolerances are derived from them, and theoretically the health and well-being of people and their environment may depend upon the proper conduct of the tests and evaluation of the data. It is therefore appropriate to examine some of the aspects of these studies, especially regarding design and interpretation. A detailed critical evaluation of all the test methods would fill volumes, but a few points have been the subject of much discussion and bear summarizing here.

First, a brief mention is in order of how the actual test systems used have become established. Although protection of the public's health, food supply and environment from harmful amounts of pesticides has been provided for by legislation, the specific test methods employed in toxicology to provide data for safety evaluation have evolved through the years according to "the state of the art". Usually the test methods, both in their general approach and specific details, have been adopted as a result of recommendations and guidelines issued by a regulatory agency, either USDA, the FDA, or currently the EPA. They are not legislation or "law", but although allegedly this is not the intent, they become *de facto* minimum standards, and when another experimental approach seems equally or more appropriate, it must be undertaken in addition to, and not instead of, the official decree. Because of this shift from recommendation to required standard, newly proposed official guidelines should be, and usually are, challenged if considered inordinately restrictive,

scientifically unsound or impractical, by experienced members of the toxicological community. Unfortunately, the track record of such challenges has been very poor.

Several factors in the design and conduct of repeated exposure experiments have prompted considerable discussion. First there is the selection of species of animal to be used. There are a few prerequisites regarding this. There are practical considerations, such as size, availability, ease of maintenance and handling and short lifespan. Good background information regarding normal clinical values, mortality, tumour incidence and other characteristics is absolutely essential. The ideal choice is the species that metabolizes a given chemical in the same manner as does man.

The scheme for determining this most appropriate test species has been described by various groups (*Food and Cosmetics Toxicology*, 1969; Food Protection Committee, 1970; Frazer, 1970; Frazer & Sharratt, 1969). Generally, it involves preliminary subacute studies in several species, from which data regarding both toxicity and metabolism can be obtained. Then, if not contraindicated by high toxicity, limited tests in man are undertaken for determination of metabolites in blood, urine and faeces. At this point it may be possible to make a reasonably good choice of species for longer-term studies. This is an extreme oversimplification of the problem for most cases, however. Numerous species may be tested, still without good correlative data. In the case of coumarin, ten species were examined before one was found that was similar to man in its metabolism of this compound (*Food and Cosmetics Toxicology*, 1970; Kaighen & Williams, 1969; Shilling, Crampton & Longland, 1969). If the species were an exotic one, difficult to handle or without adequate background information, the disadvantages could well override the advantage of an apparent metabolic similarity. The value of the information gained must be balanced against opposing factors.

The species commonly used in repeated toxicity testing have various advantages and drawbacks. The rat is the most widely used, for both its practicality and physiology. There is general agreement as to the value of studies in rats (Edson, Noakes & Sanderson, 1968; Frazer, 1969). The dog is the non-rodent species of choice for subacute testing, but its long lifespan renders it inappropriate for carcinogenic evaluations. This raises the point of the rationale of 2-year feeding studies in dogs. These studies are intended for evaluation of carcinogenicity and extend for the lifetime of other species. Furthermore, Weil & McCollister (1963), in comparisons of 21 long-term rat and dog studies, showed that dogs were no more sensitive than rats regarding other indications of toxicity. Therefore, it would seem logical to conduct studies of shorter duration in dogs since it would be much less expensive and just as valuable, as long as attempts are not made to ascertain carcinogenicity. The dog is susceptible to bladder carcinomas induced by certain aromatic amines, where other species have proved refractory (FDA Advisory Committee on Protocols for Safety Evaluation, 1971). In these cases, the 7-year or more experimental period is warranted. There are indications that the hamster may prove to be a satisfactory substitute.

Mice are used in chronic studies, especially those for the evaluation of carcinogenic potential. They are, however, very susceptible to a wide variety of spontaneous and induced tumours and their suitability for a reasonable carcinogenic investigation has been questioned (Grasso & Crampton, 1972).

Monkeys have received considerable attention as test animals. There is no evidence, however, that they are closer to man with regard to metabolic patterns than are rats or dogs (Committee on Problems of Drug Safety, 1969; Frazer, 1969; Noel, 1970). In fact, being herbivores, evolutionary adaptive mechanisms have no doubt left them quite unsuitable for evaluating the toxicity of many agents.

Various other animals have been used in special cases. The use of neonatal mice and hamsters has been in vogue in the last few years for carcinogenicity testing and has met with criticism, as the interpretation of results is most difficult (*Food and Cosmetics Toxicology*, 1968; Task Force on Research Planning in Environmental Health Science, 1970).

Earlier in this paper, mention was made of the standard clinical tests that are determined in subacute and chronic feeding studies. A few of these will be discussed in more detail at this point. Urine analyses are routinely conducted, although results are almost always normal, even in cases of morphological renal damage. Blood urea nitrogen, the most common determination for evaluation of renal function, is likewise very insensitive, normal levels being found even with severe renal damage (Sharratt, 1970). Measurement of bromsulphthalein (BSP) dye retention is a liver-function test routinely conducted in dogs. However, up to 50% of the functional capacity of the liver must be impaired before the dye test will indicate the damage (Cornish, 1971). There seems to be little scientific basis, then, for routinely conducting these tests. Rowe, Wolf, Weil & Smyth (1959) and Weil & McCollister (1963) compiled and compared data from a number of short- and long-term feeding studies and found, among other things, that the most efficient criteria for defining the lowest dosage level that produces an effect are body-weight gain, liver and kidney weights and pathological alterations in the liver and kidney. This is not to suggest that these should be the only criteria examined, but that certain clinical studies should be included only when indicated and not run routinely according to some "recipe". Furthermore, even if omission of these tests resulted in missing a change (however unlikely), the effect would be detected by other, more sensitive criteria, so there would be no loss of information. Weil & McCollister (1963) also found that results of 90-day studies are excellent predictors of the outcome of 2-year trials. Other investigators share the belief that studies of a few months duration will reveal the nature of important toxic effects, except for carcinogenicity (Paget, 1963 & 1968). Thus long-term studies could truly concentrate on the evaluation of carcinogenic potential, rather than be cluttered up with meaningless and repetitious data.

The selection of dosage levels administered in repeated dose studies is an important decision. Doses for subacute studies may be estimated from LD_{50} data, but this is often not very successful. Weil, Woodside, Bernard & Carpenter (1969) have shown short-term (7-day) tests to be much more efficient in predicting optimal dose ranges for longer term studies. In well-designed toxicological investigations, a range of doses is administered so that dose-related effects will be evident and a safe threshold dose can be calculated. The establishment of this dose-response relationship is "fundamental to all toxicological endeavour" (Golberg, 1971).

There are those in the field who advocate the administration of massive doses, presumably in an attempt to maximize the likelihood of toxic manifestations. One wonders if this might be partly due to an aversion to negative data. Striking results attract much more attention, both from certain scientific publications and from the lay media. However, the effects from excessive doses may simply be evidence of abnormal metabolism as a result of overloading the system, and are not necessarily reflections of inherent toxicity or characteristic of changes occurring at lower doses (Food Protection Committee, 1959). It is likely in many instances that normal metabolism could handle the chemical in more reasonable quantities (*idem*, 1970). Data obtained from animals given massive, unrealistic doses are especially inappropriate for assessing hazard in the light of the current emphasis on the effects of exposure to very low levels of pesticides over extended periods, perhaps a lifetime.

The practice of administering massive doses really becomes a serious problem when a

zero tolerance is applied on the basis of the results. This is true in the case of suspected carcinogens, as provided by the Delaney Clause, which states that no level of an additive is safe if it is found to induce cancer in man or animals by appropriate test procedures. This sounds like a noble pronouncement, until one realizes that the qualification in the clause relating to appropriateness is often ignored in designing experiments and interpreting results. A definition of appropriateness is definitely in order, so that our food supply will not be jeopardized by results from poorly designed, irrelevant toxicity studies.

Although not included in the Delaney Clause, the concept of "zero tolerance" has been extended by some people to cover agents that have been shown to be teratogens and mutagens, regardless of dosage, in animal studies. Various factors which may influence foetal development are notorious. Golberg (1971) has summarized these, which include transport of mice by air on days 12 and 13 of pregnancy, fasting at a critical stage or a diet of raisins for one day, along with other stress factors. Interpretation of results from mutagenicity studies is in an extremely naïve stage, with really no understanding of how the findings might apply to man. This is not to suggest that these new test systems be abandoned or ignored. However, before high-impact decisions are reached on the basis of the results, more progress should be made in evaluating the significance of findings, including consideration of dose–response relationships.

Since many of the problems in toxicity studies arise in the interpretation of data rather than in methodology, a few more words on this subject are in order here. Reverence is often made to "the level of no effect" defined by toxicity investigations, implying that thousands of dollars and sometimes years of effort have been reduced to a single number. Indeed the significance of this expression should not be minimized; not only is it of practical importance in that it is used in calculations of safety factors and tolerances, but behind it lies the primary concept in toxicology—that there is a safe level of virtually any material, below which "there is practical certainty that injury will not result . . ."; this is safety, as defined by the Food Protection Committee (1959). Arriving at a no-effect level for a given compound requires judgement based on broad experience in toxicology. The concept of the term is a dynamic one, which has to be re-evaluated with each new, more sensitive test system. One of the major interpretative problems in toxicology is that of evaluating the significance of subtle changes detected by various criteria. How these are interpreted has a definite influence on the final no-effect level defined. One of the most obvious examples of this kind of change is that of increased liver weights. Chapters have been written regarding the significance of this, and there is still some lack of agreement, although the general consensus seems to be that, in the absence of functional and morphological changes in the liver, this effect is without toxicological significance.

There are other examples of interpretative problems, but space does not permit elaboration. In general though, dilemmas still exist in distinguishing between "response and injury; no harmful effect and no detectable effect; adaptation and damage; permanent and transient damage; an acceptable and an unacceptable positive finding . . ." (Frazer, 1969).

Along with the enormous task of solving some of the interpretative problems just mentioned, there are other situations that warrant high-priority attention. More consideration should be given to naturally occurring toxins; their contribution to the exposure level of carcinogens, mutagens and teratogens should be explored. The benefits of pesticides should be weighed very carefully against the risk involved. The production of food without suitable pesticides is a frightening prospect and hasty decisions should be avoided except in a case of a real public health hazard.

Conclusions

It is very clear from this information that obtaining toxicity data on pesticides for evaluation of safety is an expensive, time-consuming procedure. Moreover, the impact of the results is considerable in that tolerances are derived from them and theoretically the health and well-being of people and their environment may depend upon the proper conduct of the test and evaluation of the data.

Often overlooked are the conservative judgements made in establishing a safe level for a pesticide. After a "no-effect" level of a pesticide for laboratory animals is established, a safety factor of 100 is frequently used in extrapolating to a safe level of intake for man, a level commonly designated as the ADI, the Acceptable Daily Intake. A tolerance level for a given pesticide established by regulation takes into consideration not only the projected safe level, but also the actual residue levels that result from the prescribed use in various commodities and the consumption patterns of the individual commodities.

Although some of the tests in question may be scientifically valid in themselves, they are inappropriately designed and/or interpreted to enhance the establishment of safety. Many procedures in toxicological testing are not only costly but redundant. Even more disturbing is the fact that many of the redundant procedures are less sensitive than others for detecting an untoward effect. These low-yield, high-cost procedures are performed to satisfy whims rather than to provide data for safety evaluation.

Currently, these policies are at the expense of the pesticide manufacturers and therefore ultimately the consumer. Hopefully, they will not render the pesticide business so prohibitively expensive and the business risk so great that the food supply of the nation and the world will suffer.

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REPEATED DERMAL APPLICATION TO RABBITS

ANIMALS: Fifty albino rabbits (New Zealand white), two to three kilograms in body weight, will be placed on experiment as follows:

Group	Number per Group	Skin Condition	Dosage
I	2 males, 2 females	Intact	Control
II	5 males, 5 females	Intact	High
	5 males, 5 females	Abraded	
III	5 males, 5 females	Intact	Low
	5 males, 5 females	Abraded	

COMPOUND ADMINISTRATION: Applied dermally to the prepared area of the back of each treated rabbit, once daily, five days per week for a period of three weeks (15 applications). Appropriate amounts of the formulation vehicle will be applied to the backs of the control rabbits in the same manner. After application, the exposed area will be covered with loosely fitting gauze to minimize the possibility of compound ingestion. All residual material will be removed prior to each daily application.

DURATION: Three weeks.

CLINICAL OBSERVATIONS: General observations daily; body weights and physical condition weekly.

HEMATOLOGY: Hemograms consisting of hemoglobin, hematocrit, and total and differential leucocyte counts to be conducted on all animals initially and at termination.

CHOLINESTERASE INHIBITION: Both RBC and plasma determinations to be run on all animals initially and at termination.

NECROPSY: To be performed on any animal dying or at the point of death and all survivors sacrificed at termination. All important organs and tissues will be examined and sections of liver, kidney, thyroid, gonads, adrenals, and application sites will be taken and preserved in 10 per cent formalin.

HISTOPATHOLOGY: The above named tissues will be examined in respect of all control and high level animals. Histopathological examination of tissues from low level animals will be conducted if indicated.

REPEATED ORAL ADMINISTRATION TO MALLARD DUCKS

PROCEDURE: Approximately 200 five-day-old mallard ducks will be used to conduct this study. A sufficient number of graded dose levels of the test materials (technical and formulation) will be administered in the diet to groups of 10 birds for seven days. The birds will be held on basal ration for an additional three days, then sacrificed and representative birds examined for gross pathological changes. Untreated control groups will be maintained in a similar manner.

Using such parameters as mortality, gross pathological changes, signs of intoxication, food consumption, and body weight changes, the possible hazard of the test material will be evaluated.

ACUTE ADMINISTRATION TO SUNFISH

PROCEDURE: Bluegill sunfish will be acclimated for a minimum of seven days. During this acclimation period, the fish will be fed Purina Trout Chow daily. For each test material, a population of approximately 110 fish of uniform size (approximately 1.5 grams in weight and 4.0 cm in length) will be selected and fasted for 72 hours.

The water in which the fish are held during the period will be deionized tap water, reconstituted by adding $CaSO_4$ (30 mg/l), $MgSO_4$ (30 mg/l), $NaHCO_3$ (48 mg/l), and KCl (3 mg/l). The water will be aerated prior to the test period.

Each test material will be dissolved in a suitable solvent and aliquots will be pipetted into test jars containing 15 liters of reconstituted water to attain desired levels of the test material in terms of parts per million (ppm).

A sufficient number of groups of 10-20 fish per group will be placed in the jars and exposed to the graded levels of the test material for a period of 96 hours.

Observations will be made at regular intervals for signs of intoxication and mortality. An LC_{50} for each test material will be determined at 48 and 96 hours.

TERATOLOGICAL STUDY IN RATS

ANIMALS: Sixty pregnant female albino rats (day of mating established) will be placed on experiment as follows:

Group	Number per Group	Dosage Level*
I	20	Control
II	20	High
III	20	Low

* Actual dose levels to be established

COMPOUND ADMINISTRATION: The test material will be administered in the diet to the Group II and III rats from day 6 to day 15 of the gestation period.

PROCEDURE: All females will be weighed on days 0, 6, 15, and 20 of gestation and food consumption recorded during the dosing period.

The numbers of fetuses, their placement in the uterine horns, correlation with the numbers of corpora lutea, live and dead fetuses, and resorptions will be determined.

All fetuses will be weighed and examined closely for externally observable signs of abnormality. One-third of the fetuses will be examined for visceral abnormalities and two-thirds cleared, stained, and examined for skeletal abnormalities.

The data are to be reported in the following format:

Observations Made	Results		
Dose Levels			
Average Body Weight of Dams(g)			
Mating Day 1			
Gestation Day 0			
Gestation Day 6			
Gestation Day 15			
Gestation Day 20			
Matings Resulting in Conceptions			
Average Fetal Weight(g)			
Average Litter Weight(g)			
Total Implantation Sites			
Average Implantation Sites/Litter			
Implantation Sites L ft Horn			
Implantation Sites Right Horn			
Total Viable Fetuses			
Total Dead Fetuses			
Total Resorption Sites			
Per Cent Resorptions			
Gross Abnormalities			
Stained Skeletal Abnormalities			
Bouin's Section Abnormalities			

TERATOLOGICAL STUDY IN RABBITS

ANIMALS: Female albino rabbits of breeding age will be acclimated to laboratory conditions and placed on experiment as follows:

Group	Pregnant Females Per Group	Dosage Level
I	10	Control
II	10	High
III	10	Low

A sufficient number of untreated adult male rabbits will be used for mating, or alternatively artificial insemination will be used.

COMPOUND ADMINISTRATION: The test material will be administered in the diet to the Group II and III animals from day 6 to day 18 of gestation.

OBSERVATIONS: All females will be weighed on days 0, 6, 12, 18, and 30 of gestation. Food consumption will be recorded during the dosing period.

On day 30 of the gestation period, all pregnant females will be sacrificed and the fetuses delivered by Caesarean section. The ovaries and uterus of each parent female will be examined for corpora lutea, implantation sites, resorptions, and other evidence of abnormal reproductive processes.

The numbers of viable and non-viable fetuses will be recorded. All surviving offspring from each litter will be placed in an incubator by litter groups for observation of 24-hour survival. All surviving fetuses will be sacrificed and these, along with all non-survivors, will be examined closely for externally observable signs of abnormality. The fetuses will then be subjected to detailed necropsy and examined for any evidence of visceral abnormality, then cleared, stained, and examined for evidence of skeletal abnormalities.

The data are to be reported in the following format:

Observations Made	Results		
Dose Level	_____	_____	_____
Maternal Mortality	_____	_____	_____
Average Body Weight of Dams(kg)			
Day 0	_____	_____	_____
Day 6	_____	_____	_____
Day 12	_____	_____	_____
Day 18	_____	_____	_____
Day 30	_____	_____	_____
Percent Conception	_____	_____	_____
Percent Alive at C-section	_____	_____	_____
Percent Resorption	_____	_____	_____
Percent Dead	_____	_____	_____
Average Litter Size	_____	_____	_____
Average Viable Litter Weight (g)	_____	_____	_____
Average Pup Weight (g)	_____	_____	_____
Incidence of Abnormalities (%)	_____	_____	_____

ACUTE INHALATION EXPOSURE TO RATS

PROCEDURE: Groups of 10 young adult albino rats each will be placed in a hynamic exposure chamber and exposed to the test material as the formulation at different levels in an effort to establish the approximate LC_{50} of the test material. The amount of exposure will be varied by chamber concentration of the test material or time of chamber exposure of the animals.

Following each exposure, the animals will be removed and the number of survivors, signs of respiratory involvement, and eye irritation noted and recorded. The survivors will be observed daily thereafter for 14 days or until death. At 14 days, all survivors will be sacrificed.

Gross necropsy will be performed on all animals dying as well as sacrificed survivors at termination. Important organs will be weighed and grossly examined. Sections of major tissues will be preserved in 10% formalin in the event histopathological observations are indicated.

SINGLE ORAL ADMINISTRATION TO RATS

PROCEDURE: Approximately 30 male and 30 female young adult albino rats each will be used to estimate the LD_{50} of the test material.

Following administration of graded doses of the test material, the animals will be observed closely for pharmacologic and toxicologic signs throughout the day. All rats will be closely observed, daily thereafter, for a period of 14 days or until death for signs of delayed toxicity. All rats that die during the observation period and representatives of survivors sacrificed at termination will be subjected to gross necropsy. Weights at 0, 4, 9, and 14 days will be recorded.

Calculations of the LD_{50} will be determined by the method of H. J. Horn (Biometrics, 12:311, 1956).

Alternate Method: Computer calculated Probit Analysis, D. J. Finney (Cambridge University Press, 1952).

SINGLE ORAL ADMINISTRATION TO RABBITS

PROCEDURE: Sixteen to twenty young adult rabbits (New Zealand white) will be used to estimate the LD_{50} of the test material.

Following administration of graded doses of the test materials, the animals will be observed closely for pharmacologic and toxicologic signs throughout the day. All rabbits will be observed closely, daily thereafter, for a period of 14 days or until death for signs of delayed toxicity. All rabbits that die during the observation period and representatives of survivors sacrificed at termination will be subjected to gross necropsy. Weights at 0, 4, 9, and 14 days will be recorded.

Calculations of the LD_{50} will be determined by the method of H. J. Horn (Biometrics, 12:311, 1956).

Alternate method: Computer calculated Probit Analysis, D. J. Finney (Cambridge University Press, 1952).

SINGLE ORAL ADMINISTRATION TO DOGS

PROCEDURE: Six to eight normal mongrel dogs will be used to estimate the approximate lethal dose of the test material when administered.

Following administration, the animals will be observed closely for pharmacologic and toxicologic signs throughout the day. All dogs will be observed closely, daily thereafter, for a period of 14 days or until death for signs of delayed toxicity. All dogs that die during the observation period will be subjected to gross necropsy. Weights at weekly intervals, two weeks prior to and two weeks following compound administration will be recorded.

REPEATED ORAL ADMINISTRATION TO RATS

FOR 13 WEEKS

ANIMALS: One hundred twenty albino rats obtained at weaning from The Charles River Breeding Laboratories, Inc., housed individually in temperature-controlled quarters, and acclimated to laboratory conditions for one week will be placed on experiment as follows:

Group	Number per Group	Dosage Level*
I	15 males, 15 females	Control
II	15 males, 15 females	High
III	15 males, 15 females	Intermediate
IV	15 males, 15 females	Low

* Actual dose levels to be established

COMPOUND ADMINISTRATION: By incorporation in the diet by mechanical mixer with levels adjusted to maintain a relatively constant intake of test material in terms of mg/kg/day.

DURATION: Thirteen weeks

CLINICAL OBSERVATIONS: General observations daily; body weights, food consumption, and physical examination weekly.

HEMATOLOGY: Hemograms consisting of hemoglobin, microhematocrit, coagulation time, thrombocyte counts, and total and differential leucocyte counts will be determined on 5 males and 5 females each from Groups I and II at 0, 4,

8 and 13 weeks. Lower levels will be examined when indicated by results in Group II.

CLINICAL CHEMISTRY: Blood glucose, prothrombin time, and serum glutamic pyruvic transaminase will be determined on 5 males and 5 females each from Groups I and II at 0, 4, 8 and 13 weeks. Lower levels will be examined when indicated by results in Group II.

NECROPSY: To be performed on any animal dying or at the point of death and all survivors sacrificed at termination.

The following organs will be weighed: heart, lung, liver, kidneys, spleen, gonads, adrenal, thyroid, prostate or uterus, and pituitary. Portions of these organs plus portions of the following tissues will be grossly examined and preserved in 10 percent formalin: duodenum, intercostal muscle, urinary bladder, pancreas, mesenteric lymph node, mammae, bone marrow, stomach, and brain. In addition, eyes will be taken, grossly observed, and preserved in Zenker's fixative.

HISTOPATHOLOGY: The above named tissues (approximately 18 per rat) will be examined from any animal dying when the tissues are in good condition and from 5 males and 5 females each from Groups I and II sacrificed at termination. In addition, eight selected tissues will be examined from 5 males and 5 females each from Groups III, IV and V sacrificed at termination. Additional tissues will be examined from lower levels if indicated by results from Group II.

REPEATED ORAL ADMINISTRATION TO DOGS (OR MONKEYS) FOR 13 WEEKS

ANIMALS: Twenty-four purebred beagle dogs, six to eight months of age, individually housed in temperature-controlled quarters, properly treated for intestinal parasites, immunized against rabies, distemper, hepatitis, and leptospirosis, and acclimated to laboratory conditions for three weeks will be placed on experiment as follows:

Group	Number per Group	Dosage Level*
I	3 males, 3 females	Control
II	3 males, 3 females	High
III	3 males, 3 females	Intermediate
IV	3 males, 3 females	Low

* Actual dose levels to be established

COMPOUND ADMINISTRATION: By gelatin capsule, once daily, seven days per week, with empty gelatin capsules to controls.

DURATION: Thirteen weeks

CLINICAL OBSERVATIONS: General observations consisting of behavior, food intake, stool consistency, etc., daily; body weights and detailed physical examination weekly. In addition, heart rate and blood pressure will be determined and electrocardiograms and ophthalmic examinations will be conducted on all animals initially and at 4, 8 and 13 weeks.

HEMATOLOGY: Hemograms consisting of hemoglobin, hematocrit, sedimentation rate, coagulation time, thrombocyte counts, and total and differential leucocyte counts will be determined on all animals twice initially and at 4, 8 and 13 weeks.

CLINICAL CHEMISTRY: Blood urea nitrogen, serum alkaline phosphatase, blood glucose, prothrombin time, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminase will be determined on all animals twice initially and at 4, 8 and 13 weeks.

URINALYSES: Qualitative urinalyses consisting of general appearance, pH, specific gravity, albumin, glucose, and microscopic examination of urinary sediment will be determined on cage-collected samples from all animals initially and at 4, 8 and 13 weeks.

NECROPSY: To be performed on any animal dying or at the point of death and all survivors sacrificed at termination.

The following organs will be weighed: heart, lungs, liver, kidneys, spleen, thyroid, adrenal, prostate or uterus, gonads, pituitary, and brain. Portions of these organs plus portions of the following tissues will be grossly examined and preserved in 10 per cent formalin: nerve, esophagus, duodenum, jejunum, ileum, colon, cecum, stomach, pancreas, parotid salivary gland, thymus, trachea, gall bladder, intercostal muscle, urinary bladder, mesenteric lymph node, femoral bone

marrow, spinal cord, mammae, and abdominal skin. In addition, eyes will be taken, grossly observed, and preserved in Zenker's fixative.

HISTOPATHOLOGY: The above named tissues (approximately 32 per animal) will be examined from any animal dying when the tissues are in good condition and from all control and high level animals. In addition, 13 selected tissues will be examined for each of the remaining animals. Additional tissues will be examined from lower levels if indicated by results from Group II.

THREE-GENERATION REPRODUCTION STUDY IN THE RAT

ANIMALS: One hundred twenty albino rats, obtained at weaning from The Charles River Breeding Laboratories, Inc., will be acclimated to laboratory conditions for one week and placed on experiment as follows:

Group	Number per Group	Dosage Level*
I	20 males, 20 females	Control
II	20 males, 20 females	High
III	20 males, 20 females	Low

* Actual dose levels to be established

COMPOUND ADMINISTRATION: By incorporation in the diet. The experimental material will be administered at appropriate levels of concentration to all treated animals and their surviving offspring beginning one week after weaning.

PROCEDURE:

1. When the above animals (F_0 generation) are 100 days of age, they will be paired to mate by placing a female in a corresponding male's cage for a period of 10 days.

2. All offspring from this mating (F_1a generation) will be taken as soon after birth as possible, weighed, and examined for externally observable signs of abnormality. One-half of each litter of the new born will be preserved in Bouin's solution and examined for visceral abnormalities an one-half will be cleared, stained, and examined for skeletal abnormalities. Stillborn animals will be similarly examined.

3. Immediately following the birth of the F_1a generation animals, the F_0 females will be mated, this time with a different male, producing the F_1b generation. Each litter will be reduced to 10 pups on day 5 and all offspring will be weighed and examined on days 1, 5, and 21. The number of dead pups at birth, days 1-5, and 6-21 will be recorded. Following the weaning of these offspring, the parent animals will be sacrificed and subjected to gross necropsy. Gonads will be weighed.

4. One or two animals of each sex from each F_1b generation litter will then be selected to give 20 males and 20 females per dosage level and the remaining weanlings sacrificed.

5. These animals will be mated as before, producing an F_2a generation. The offspring will be weighed and observed as described under Item 3 above and sacrificed at weaning.

6. Ten days after the sacrifice of the F_2a weanlings, the F_1b females will be remated, this time with a different male, producing the F_2b generation. These offspring will be weighed and observed, and the parent (F_1b) animals sacrificed and necropsied, as described under Item 3 above.

7. One or two animals of each sex from each F_2b generation litter will then be selected to give 20 males and 20 females per dosage level and the remaining weanlings sacrificed.

8. These animals will be mated as before, producing an F_3a generation. The offspring will be weighed and observed as described under Item 3 above and sacrificed at weaning.

9. Ten days after the sacrifice of the F_3a weanlings, the F_2b females will be remated, this time with a different male, producing an F_3b generation. These offspring will be weighed and observed, and the parent (F_2b) animals sacrificed and necropsied, as described under Item 3 above.

10. At this time all F_3b weanlings will be sacrificed and subjected to gross necropsy. Heart, liver, and kidneys from two pups per sex per litter will be weighed. Portions of these plus portions of spleen, adrenals, thyroid, gonads, and bone marrow from all F_3b animals will be taken and preserved in 10 per cent formalin.

11. The above named tissues will be examined histopathologically from one male and one female per each F_3b generation litter; tissues will be examined from more animals, if indicated.

TEST FOR EYE IRRITANTS

Six albino rabbits are used for each test substance. Animal facilities for such procedures shall be so designed and maintained as to exclude sawdust, wood chips, or other extraneous materials that might produce eye irritation. Both eyes of each animal in the test group shall be examined before testing, and only those animals without eye defects or irritation shall be used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. For testing liquids, 0.1 milliliter is used. For solids or pastes, 100 milligrams of the test substance is used, except that for substances in flake, granule, powder, or other particulate form the amount that has a volume of 0.1 milliliter (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) shall be used whenever this volume weighs less than 100 milligrams. In such a case, the weight of the 0.1 milliliter test dose should be recorded. The eyes are not washed following installation of test material except as noted below.

The eyes are examined and the grade of ocular reaction is recorded at 24, 48 and 72 hours. Reading of reactions is facilitated by use of a binocular loupe, hand slit-lamp, or other expert means. After the recording of observations at 24 hours, any or all eyes may be further examined after applying fluorescein. For this optional test, one drop of fluorescein sodium

ophthalmic solution USP or equivalent is dropped directly on the cornea. After flushing out the excess fluorescein with sodium chloride solution USP or equivalent, injured areas of the cornea appear yellow; this is best visualized in a darkened room under ultraviolet illumination. Any or all eyes may be washed with sodium chloride solution USP or equivalent after the 24-hour reading.

An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than a fine stippling), or opacity of the cornea (other than a slight dulling of the normal luster), or inflammation of the iris (other than a slight deepening of the folds (or rugae) or a slight circumcorneal injection of the blood vessels), or if such substance produces in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids of a diffuse crimson-red with individual vessels not easily discernible.

The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test shall be regarded as negative. If two or three animals exhibit a positive reaction, the test is repeated using a different group of six animals. The second test shall be considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test shall be repeated with a different group of six animals. Should a third test be needed,

the substance will be regarded as an irritant if any animal exhibits a positive reaction.

To assist testing laboratories and other interested persons in interpreting the results obtained when a substance is tested in accordance with the method described in the first paragraph, an "Illustrated Guide for Grading Eye Irritants by Hazardous Substances" will be sold by the Superintendent of Documents, Government Printing Office, Washington, D. C. The guide will contain color plates depicting responses of varying intensity to specific test solutions. The grade of response and the substance used to produce the response will be indicated.

EVALUATION OF CARCINOGENIC POTENTIAL IN RATS BY REPEATED ORAL ADMINISTRATION

ANIMALS: Four hundred eighty albino rats obtained at weaning from The Charles River Breeding Laboratories, housed individually in temperature-controlled quarters, and acclimated to laboratory conditions for one week will be placed on experiment as follows:

Group	Number per Group	Dosage Level*
I	60 males, 60 females	Control
II	60 males, 60 females	High
III	60 males, 60 females	Intermediate
IV	60 males, 60 females	Low

* Actual dose levels to be established

DURATION: Approximately two years (life span of test animals).

COMPOUND ADMINISTRATION: By incorporation in the diet by mechanical mixer with levels adjusted to maintain a relatively constant intake of test material in terms of mg/kg/day.

CLINICAL OBSERVATIONS: General observations daily; food consumption weekly; body weights and detailed physical examination by biweekly intervals.

HEMATOLOGY: Hemograms consisting of hemoglobin, hematocrit, sedimentation rate, coagulation time, thrombocyte counts, and total and differential leucocyte counts will be determined on five males and five females each from Groups I and II at 3, 6, 12, 18 and 24 months.

CLINICAL CHEMISTRY: Blood urea nitrogen, serum alkaline phosphatase, blood glucose, prothrombin time, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminase (others as indicated) will be determined on five males and five females each from Groups I and II at 3, 6, 12, 18 and 24 months. In addition, plasma and RBC cholinesterase will be determined initially and at 3, 6, 12, 18 and 24 months and brain cholinesterase at termination if indicated.

URINALYSES: Qualitative urinalyses consisting of general appearance, pH, specific gravity, albumin, glucose and microscopic examination of urinary sediment will be determined on cage-collected samples from five males and five females each from Groups I and II at 3, 6, 12, 18 and 24 months.

NECROPSY: To be performed on any animal dying or at the point of death. Portions of the following organs and tissues will be grossly examined and preserved in 10 per cent formalin: heart, lung, liver, kidneys, spleen, gonads, adrenal, thyroid, prostate or uterus, pituitary, duodenum, intercostal muscle, urinary bladder, pancreas, mesenteric lymph node, bone marrow, stomach, brain, eyes and tissue mass. A sample of brain tissue will be taken from five males and five females each from Groups I and II and frozen for cholinesterase determinations if indicated.

HISTOPATHOLOGY: Any suspected abnormal organ or tissues will be examined with respect to all animals.

END